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Nasal carriage and antimicrobial susceptibility of *Staphylococcus aureus* among medical students at the HRH Princess Maha Chakri Sirindhorn Medical Center, Thailand: A follow-up study

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KEYWORDS
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Summary
Objective: The objective of this study is to evaluate the patterns of nasal colonization of *Staphylococcus aureus* and its susceptibility patterns among medical students before and after their rotations in the hospital.
Methods: Nasal swabs were obtained from 128 medical students for microbiological study and susceptibility testing prior to working in the hospital (the first), following the first rotation (the second) and at the end of the rotation schedule in the hospital (the last). The probable risk factors for nasal carriage were recorded for assessment.
Results: *S. aureus* was isolated at the first, second and last swabs with colonization rates of 29.7%, 30.5% and 39.4%, respectively. The prevalence rate of colonization of *S. aureus* showed a statistically significant increase ($P < 0.05$). There was a persistent colonization of *S. aureus* at the rate of 20.3%. No participants showed methicillin-resistant *S. aureus*. The susceptibility of *S. aureus* to erythromycin and clindamycin was 36.8%, 41% and 34% at the first, second and last swabs, respectively. There was no significant correlation between nasal carriage of *S. aureus* and its potential risk factors.
Conclusions: After clinical rotation in the hospital, the prevalence rate of asymptomatic nasal carriage of *S. aureus* increased and the *S. aureus* isolated has shown a relatively high resistance to erythromycin and clindamycin.

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Introduction

Staphylococcus aureus is commonly colonized in human nares. Approximately 20% of healthy subjects are identified as persistent carriers [1]. The hospitalized patients who are colonized with *S. aureus* are at risk of becoming infected with the strain found in nasal vestibules [2]. Meanwhile, most cases of nosocomial infections with *S. aureus* usually spread through the hands of health care workers. These health care workers either have been colonized with *S. aureus* in their nares or have contacted an infected patient. In the analysis of *S. aureus* infections in hospitalized patients in tertiary care centers, Methicillin-sensitive *S. aureus* (MSSA) and Methicillin-resistant *S. aureus* (MRSA) were identified in 58.5% and 41.5% of these patients, respectively [3]. Among these patients, the high incidence of antimicrobial resistance can generate treatment complexities [4]. In our previous study, 29.7% of preclinical medical students were found to be colonized with *S. aureus* prior to working in the hospital, and strains of MRSA were not detected. *S. aureus* isolates exhibited a high resistance to erythromycin and clindamycin [5].Consequently, the aim of this study is to evaluate the patterns of nasal colonization of *S. aureus* and its susceptibility patterns among medical students before and after rotation in the hospital.

Materials and methods

Study design and subjects

This study has been designed as a follow-up study. The subjects enrolled in our study are all healthy, third-year, preclinical medical students at Srinakharinwirot University, HRH Princess Maha Chakri Sirindhorn Medical Center (MSMC), Thailand. The first nasal swabs were taken in March 2012, prior to the beginning of the students' clinical rotations in the hospital. Each rotation was set for a period of eight weeks. The second nasal swabs were taken after the end of the first ward rotation. The rotation schedule is as follows: General Internal Medicine, Pediatrics, Surgery, Gynecology and Psychiatry. The last nasal swabs were taken at the end of the rotation schedule during the same calendar year. Participants were excluded if they had either a history of respiratory infections requiring hospitalization or current skin infections (or both) anytime during the four weeks prior to the collection of the nasal swabs. Demographic data including age, gender, underlying diseases, antibiotic usage in the four-week period prior to collection, hand-washing habits, bathing habits, the sharing of personal belongings, and ward rotations were recorded for the assessment as potential risk factors. The research was reviewed and approved by the appropriate institutional review boards and the ethics committee of the MSMC. All participants had provided letters of consent before engaging in this study.

Samples collection and antimicrobial susceptibility testing

Swabs of the anterior nasal vestibules of the subjects were collected and processed at the MSMC microbiology laboratory. Standard microbiological procedures were used to isolate, identify and determine the numbers of the organisms by using 5% sheep blood agar, chocolate agar, mannitol salt agar and MacConkey's agar. The microorganisms were identified using colony forming morphology on culture plates, Gram staining, catalase production and tube coagulase tests.

Susceptibility testing of *S. aureus* was performed using Kirby Bauer's disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines from 2012 [6]. The following antibiotics were used: ciprofloxacin (5 mcg), gentamycin (10 mcg), chloramphenicol (30 mcg), co-trimoxazole (25 mcg), tetracycline (30 mcg), erythromycin (15 mcg), clindamycin (2 mcg), fosfomycin (50 mcg), fusidic acid (10 mcg) and linezolid (30 mcg). *S. aureus* (ATCC 25923) was used as the control strain. A cefoxitin disk diffusion test was employed for the detection of MRSA, according to the CLSI guidelines of 2012 [6]. Inducible clindamycin resistance was detected by the double disk diffusion test (D test), which was conducted by placing clindamycin and erythromycin disks 15 mm apart. Supplementary antimicrobial discs were used for gram-negative bacteria susceptibility testing. These included ampicillin (10 mcg), amoxicillin/clavulanic acid (20/10 mcg), cefuroxime (30 mcg), cefoxitin (30 mcg), cefotaxime (30 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), cefepime (30 mcg), cefoperazone/sulbactam (75/10 mcg), imipenem (10 mcg), meropenem (10 mcg), ertapenem (10 mcg) and piperacillin/tazobactam (100/10 mcg).

Statistical analysis

Statistical analysis was performed using SPSS from IBM Singapore Pte Ltd (Registration No. 1975-01566-C). The findings were analyzed using descriptive statistics. The relationship between each variable

Table 1 Epidemiological profile and feasible risk factors for *S. aureus* colonization.

Variables	*S. aureus*			OR (95%CI)	*P*
	Total No. (%)	Positive No. (%)	Negative No. (%)		
Gender					
Male	53 (41.8)	23 (43.4)	30 (56.6)	1.36 (0.66–2.79)	0.39
Female	74 (58.2)	27 (36.5)	47 (63.5)		
Underlying diseases					
Yes	47 (37.1)	17 (36.2)	30 (63.8)	0.82 (0.39–1.73)	0.61
No	80 (62.9)	33 (41.2)	47 (58.8)		
Antibiotic usage in the last four weeks					
Yes	22 (17.3)	8 (36.4)	14 (63.6)	0.89 (0.34–2.31)	0.81
No	105 (82.7)	41 (39.0)	64 (61.0)		
Hand washing					
Regular	29 (22.8)	10 (34.5)	19 (65.5)	0.78 (0.32–1.84)	0.57
Irregular	98 (77.2)	40 (40.8)	58 (59.2)		
Bathing habits					
Regular	104 (81.9)	41 (39.4)	63 (60.6)	1.08 (0.43–2.71)	0.86
Irregular	23 (18.1)	9 (39.1)	14 (60.9)		
Sharing of personal belongings					
Regular	22 (17.3)	9 (40.9)	13 (59.1)	1.09 (0.43–2.79)	0.84
Irregular	105 (82.7)	41 (39.0)	64 (61.0)		
Ward rotation[a]					
Psychiatry	24 (18.8)	10 (41.7)	14 (58.3)	2.48 (0.74–8.35)	0.14
Surgery	26 (20.5)	10 (38.5)	16 (61.5)	1.98 (0.60–6.64)	0.27
Pediatrics	26 (20.5)	11 (42.3)	15 (57.7)	2.32 (0.69–7.74)	0.17
Internal medicine	26 (20.5)	12 (46.2)	14 (53.8)	2.71 (0.82–9.00)	0.11
Gynecology	25 (19.7)	6 (24.0)	19 (76.0)	1	–

OR: odds ratio, CI: confidence interval.
[a] Logistic regression was used to measure the influence of the independent variables.

and the outcome (nasal carriage of *S. aureus*) was explored using the odds ratio (OR). Associations with a *P*-value <0.05 were considered to be statistically significant.

Results

A total of 128 participants enrolled in the study, with a mean age of 20.9 ± 0.9 years. Prior to the conclusion of the study, there was one withdrawal from the study due to an academic problem. All participants were colonized with microorganisms in their nasal vestibules at the first and second swabs. From the last swabs, 3.9% of the participants showed no bacterial growth. *S. aureus* was isolated from the participants at the first, second and last swabs with colonization rates of 29.7%, 30.5% and 39.4%, respectively. The prevalence rate of *S. aureus* showed a statistically significant increase ($P < 0.05$).

Throughout the study period, the colonization of *S. aureus* was identified as the persistent carrier with a rate of 20.3%. There were new cases of *S. aureus* at the second and last swabs with rates of 7.0% and 13.3%, respectively, while 50% of participants did not show colonization with *S. aureus*. In this study, MRSA was not detected by the cefoxitin disk diffusion test. There was no significant correlation between nasal carriage with *S. aureus* and any of the feasible risk factors (gender, underlying diseases, antibiotic usage in the previous four weeks, hand washing habits, bathing habits, the sharing of personal belongings, and ward rotation, $P > 0.1$), as shown in Table 1.

The susceptibility profile analysis of *S. aureus* is shown in Table 2. No *S. aureus* resistance to gentamicin, ciprofloxacin, co-trimoxazole or linezolid was detected. At the first, second and last swabs, respectively, the susceptibility of *S. aureus* to erythromycin and clindamycin was 36.8%, 41% and 34%; to tetracycline was 65.8%, 66.7% and NA; to chloramphenicol was 97.4%, 97.4% and 100%; to fusidic acid was 94%, 97.4% and 97.4%. Inducible clindamycin resistance was also found at the first, second and last swabs with rates of 4.2%, 13.0% and 6.0% of clindamycin-resistant isolates, respectively.

In this study, the bacteria isolated from the overall nasal colonization were Coagulase negative *Staphylococci*, *Corynebacterium* spp., *Klebsiella* spp., *Citrobacter koseri*, *Enterobacter* spp., *Proteus* spp., *Pseudomonas* spp., *Escherichia coli*, *Providencia rettgeri* and *Serratia marcescens*.

Table 2 Antimicrobial susceptibility patterns of *S. aureus* from each swab.

S. aureus	The first swab			The second swab			The last swab		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ciprofloxacin	100	0	0	100	0	0	100	0	0
Gentamicin	100	0	0	100	0	0	100	0	0
Chloramphenicol	97.4	0	2.6	97.4	0	2.6	100	0	0
Co-trimoxazole	100	0	0	100	0	0	NA	NA	NA
Tetracycline	65.8	0	34.2	66.7	0	33.3	NA	NA	NA
Erythromycin	36.8	0	63.2	41.0	0	59.0	34.0	0	66.0
Clindamycin	36.8	0	63.2	41.0	0	59.0	34.0	0	66.0
Fosfomycin	97.4	2.6	0	100	0	0	100	0	0
Fusidic acid	97.4	0	2.6	97.4	0	2.6	94.0	2.0	4.0
Linezolid	100	0	0	100	0	0	100	0	0

S: sensitive, I: intermediate sensitivity, R: resistance.
NA: the susceptibility testing was not performed because of the lack of antimicrobial disk testing.

The prevalence of gram-negative bacteria had increased when compared to the first swabs. The prevalence of *Klebsiella* spp. increased from 7.8% in the first swabs to 18.8% and 14.2% in the second and the last swabs, respectively. Other gram-negative bacteria also had an increased rate, as shown in Table 3. After the participants' rotations in the hospital, drug resistant gram-negative bacteria were isolated from two participants who had no history of antibiotic use prior to being swabbed. These bacteria were *Serratia marcescens* and *Enterobacter* spp., which were resistant to Amoxicillin/Clavulanic acid, Cefuroxime and Cefoxitin.

Discussion

S. aureus has long been recognized as a virulent pathogen with nasal colonization. For health care workers, the transmission of the pathogen to susceptible patients is of major concern. In parallel with the increasing numbers of antimicrobial-resistant organisms, this has further complicated treatments. In this prospective study, the colonization rates of *S. aureus* in the students' nares were in the range of 29.7—39.4%. In this study, 20.3% of participants have shown persistent colonization of *S. aureus*, which is congruent with the report of J. Kluytmans et al. [1]. Therefore, health care workers can act as pathogen reservoirs, for resistant strains in particular, and could transmit them to the patients. Although the prevalence of MRSA isolates from clinical samples in our hospital was measured to be 47—50% (unpublished data), there no MRSA was detected in our study. Our findings are consistent with another report regarding medical students [7]. Interestingly, the study performed in China showed different findings [8]. The differences in the data indicate an epidemiological variability of nasal colonization by MRSA.

Table 3 An overview of bacterial nasal colonization from each swab.

	First swab		Second swab		Third swab	
	Number	%	Number	%	Number	%
Participants	128	100	128	100	127[a]	100
S. aureus	38	29.7	39	30.5	50	39.4
CoNS[b]	98	76.6	96	75.0	75	59.0
Corynebacterium spp.	13	10.2	29	22.6	10	7.9
Klebsiella spp.	10	7.8	24	18.8	18	14.2
Citrobacter koseri	5	3.9	9	7.0	5	3.9
Enterobacter spp.	3	2.3	4	3.1	8	6.3
Proteus spp.	1	0.8	0	0	0	0
Pseudomonas spp.	0	0	1	0.8	1	0.8
Escherichia coli	0	0	2	1.6	1	0.8
Providencia rettgeri	0	0	0	0	2	1.6
Serratia marcescens	0	0	1	0.8	3	2.4

[a] One participant's withdrawal from the study.
[b] Coagulase-negative *Staphylococci*.

However, for the prevention of transmission, medical students and all health care workers should be included in hospital infection control programs. This predominantly includes the practices of hand hygiene, surveillance agendas and the encouragement of added concern among health care workers regarding the subject of nosocomial infections.

In comparison to previous reports [5], the antimicrobial susceptibility patterns of *S. aureus* show high sensitivities to gentamicin, ciprofloxacin, cotrimoxazole and linezolid. However, high rates of resistance against erythromycin and clindamycin continue, as is consistent with previous literature. The rates of erythromycin-resistant *S. aureus* globally were at 26.7—78.9%, with variances in accordance to the different entities [9—11]. This resistance pattern might be the result of the excessive use of macrolides. The reason for the genetic resistance should be studied further.

In our study, with the exception of *S. aureus*, drug-resistant gram-negative bacteria were isolated from medical students after their rotations in the hospital. These resistant bacteria are generally related to health-care-associated infections, which could possibly spread to compromised patients. Patients infected with drug-resistant bacteria are more prone to have longer hospital stays and exorbitant hospital costs. The findings regarding these nasal colonizing organisms should be considered when outlining comprehensive prevention strategies for hospitals.

In summary, the prevalence rate of asymptomatic nasal carriage of *S. aureus* in healthy medical students after their hospital rotations increased in comparison to the first swabs. Because of the persistent colonization of *S. aureus* and the relatively high resistance to erythromycin and clindamycin, infection control professionals should conduct proper hygiene programs to help in preventing the spread of these organisms.

Conflicts of interest

Funding: No funding sources.

Competing interests: None declared.

Ethical approval: Not required.

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